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# Skeletal Sexual Dimorphism: Relative Contribution of Sex Steroids, Growth Hormone – Insulin-Like Growth Factor-I (GH-IGF-I) and Mechanical Loading

*Authors*: Filip Callewaert[1], Mieke Sinnesael[1], Evelien Gielen[1], Steven Boonen[1] and Dirk Vanderschueren[1]

[1] Center for Musculoskeletal Research, Leuven University Department of Experimental Medicine, Katholieke Universiteit Leuven, Herestraat 49, B-3000 Leuven, Belgium, Phone: +32-16345970, Fax: +32-16345934

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*Corresponding author*: Dirk Vanderschueren, Dirk.Vanderderschueren@uz.kuleuven.be

## Abstract

Structural gender differences in bone mass – characterized by wider but not thicker bones – are generally attributed to opposing sex steroid actions in men and women. Recent findings have redefined the traditional concept of sex hormones as the main regulators of skeletal sexual dimorphism. Growth hormone (GH) – insulin-like growth factor-I (IGF-I) action is likely to be the most important determinant of sex differences in bone mass. Estrogens limit periosteal bone expansion but stimulate endosteal bone apposition in females, whereas androgens stimulate radial bone expansion in males. Androgens not only act directly on bone through the androgen receptor (AR) but also activate estrogen receptor-α or –β (ERα or ERβ) following aromatization into estrogens. Both AR and ERα pathways are needed to optimize radial cortical bone expansion, whereas AR signaling alone is the dominant pathway for normal male trabecular bone development. Estrogen/ERα-mediated effects in males may – at least partly – depend on interaction with IGF-I. In addition, sex hormones and their receptors impact on the mechanical sensitivity of the growing skeleton. AR and ERβ signaling may limit the osteogenic response to loading in males and females, respectively, while ERα may stimulate the response of bone to mechanical stimulation in the female skeleton.

Overall, current evidence suggests that skeletal sexual dimorphism is not just the end result of differences in sex steroid secretion between sexes, but depends on gender differences in GH-IGF-I and mechanical sensitivity to loading as well.

## Introduction

Sex steroids are mainly synthesized by the gonads (testis and ovary), but the adrenals constitute an additional source (Vanderschueren *et al.* 2004, Callewaert *et al.* 2010a). Sex steroids are involved in the regulation of a number of important physiological processes, including sexual differentiation of the genitalia, sexual maturation and reproduction. Sex hormones also impact on skeletal homeostasis, because they add bone during puberty and subsequently maintain skeletal integrity, both in men and women (Riggs *et al.* 2002). However, gender differences in bone growth become apparent during puberty, with men reaching higher peak bone mass, greater bone size and, ultimately, a stronger skeleton compared to women (Garn 1970, Seeman 2001). Puberty builds a bigger but not a denser skeleton in males, as bone mineral acquisition in long bones occurs in proportion to the volume of the bone (Zamberlan *et al.* 1996). As a result, the volumetric BMD does not differ between men and women. In addition, growth during puberty also builds wider and slightly taller vertebral bodies in men, without sex differences in trabecular BMD (Gilsanz *et al.* 1994). As a result of these stronger structural features achieved during growth, the male skeleton is less susceptible to osteoporosis later in life (Seeman 2002). Although fewer men than women sustain fractures during aging, fragility fractures are common in men and are associated with a significant burden in terms of morbidity, mortality and economic cost to the community (Boonen *et al.* 2007, Khosla *et al.* 2008). Therefore, more insight into the mechanisms involved in bone mass acquisition is essential to improve our understanding of the pathophysiology of osteoporosis and osteoporotic fracture risk in men.

Skeletal gender differences in radial bone growth (skeletal sexual dimorphism) are traditionally attributed to stimulatory 'male' androgen action as opposed to inhibitory 'female' estrogen action on periosteal bone formation. However, particularly in men, the mechanism of action of sex steroids on bone growth appears considerably more complex. Testosterone (T) – the main circulating androgen in males – not only activates the androgen receptor (AR), but also acts on the estrogen receptor-α or -β (ERα or ERβ) following aromatization into 17β-estradiol ($E_2$) (Vanderschueren *et al.* 2004, Callewaert *et al.* 2010a). Experiments in animals as well as a number of case reports of men with either a loss-of-function mutation in ERα or aromatase deficiency provided evidence that estrogens play a key role in male skeletal homeostasis (Smith *et al.* 1994, Vanderschueren *et al.* 1997, Vidal *et al.* 2000, Bouillon *et al.* 2004, Rochira *et al.* 2007, Smith *et al.* 2008). In addition to sex steroids, other hormones such as growth hormone (GH) and insulin-like growth factor-I (IGF-I) may further contribute to the development of the skeletal sexual dimorphism. GH and IGF-I – the GH-IGF-I axis – are both primarily responsible for postnatal growth (Lupu *et al.* 2001). Moreover, sex steroids and the GH-IGF-I axis interact closely during puberty (Mauras *et al.* 1996), optimizing bone mass acquisition during pubertal growth. Finally, skeletal growth is further stimulated by mechanical loading (Frost 2003), which in turn may be influenced by sex hormones as well. In fact, mechanical loading increases bone formation in close association with estrogen signaling, at least in female mice (Lee *et al.* 2003). Thus, sex steroids, IGF-I and mechanical stimuli may both independently and mutually affect the acquisition of an optimal bone mass during puberty. In this review three major questions will be addressed: (1) To what extent do sex steroids or GH-IGF-I or both influence the skeletal gender

differences; (2) What is the relative importance of AR and ERα signaling in the acquisition of male cortical and trabecular bone mass; (3) Do sex steroids and their receptors affect the adaptive response of bone to loading?

## Hormonal factors involved in the development of the skeletal sexual dimorphism

### *Evidence for a role of androgens and estrogens*

Puberty represents a critical growth period during which important gender differences in bone width and strength are established. In fact, boys develop a larger periosteal perimeter than girls from midpuberty onward (Seeman 2001, Kirmani *et al.* 2009). In contrast, girls experience less periosteal expansion but more endocortical apposition compared to boys. As a result, men not only build up wider bones but also stronger bones, with cortical bone further away from the neutral axis of the long bone and more resistant to bending. Sex hormones have traditionally been considered the primary mediator of skeletal sexual dimorphism in bone size and strength. This prevailing opinion was established by the observation of a reduced periosteal perimeter in orchidectomized growing male rats versus an increased periosteal circumference in ovariectomized female rats (Turner *et al.* 1990). This landmark study led to the assumption that androgens were stimulatory and estrogens inhibitory for male and female radial bone growth, respectively. In line with this concept, a study in pubertal mouse models showed that androgen withdrawal – as induced by orchidectomy – decreases radial bone expansion in males (Callewaert *et al.* 2010b). Ovariectomy, on the other hand, increases radial bone

growth in females (Callewaert *et al.* 2010b). However, these hormonal effects differ in their timing of action, as the androgen-mediated effects occur only during later stages of puberty, compared to the earlier effect of estrogens and are in line with the traditional concept that androgens stimulate male bone size whereas estrogens limit female bone size. However, recent findings have partly redefined this concept, with a significant body of evidence pointing to a role of estrogen action in males as well. In male mice, estrogen deficiency on top of androgen withdrawal in male mice further reduces radial bone expansion, at least during the early stages of puberty (Callewaert *et al.* 2010b), in line with the concept that aromatization of androgens in estrogens also contributes to the skeletal gender differences (Figure 1).

*Evidence for a critical role of GH-IGF-I*

Other hormones such as GH and IGF-I also manifestly increase during puberty and are regarded as critical regulators of pubertal bone growth as well (Mauras *et al.* 1996). Importantly, GH-IGF-I action even appears to be the most important determinant of gender differences in bone mass in pubertal mice (Callewaert *et al.* 2010b). IGF-I levels are indeed higher in male versus female mice during early puberty, the time window during which most of the gender differences are established. Moreover, mice with a disrupted GH receptor (GHR) – associated with extremely low IGF-I levels – also have a severely reduced radial bone expansion without gender differences in radial bone growth (Callewaert *et al.* 2010b). Earlier observations of severe growth retardation in mice lacking GHR, IGF-I or both support the crucial role of GH and IGF-I in the control of

postnatal bone growth (Lupu *et al.* 2001) (Figure 1). Human and animal studies agree with this concept, as IGF-I treatment in GHR knockout (KO) mice or patients with GH resistance stimulates growth or even reverses the detrimental effects of GHR deficiency (Laron 1999, Sims *et al.* 2000). Beside this direct action of GH/IGF-I, however, there is also ample evidence for an interacting role of sex steroids and IGF-I. For instance, the pubertal increase in GH-IGF-I appears to be mediated by sex steroids. Neonatal T secretion establishes the GH secretion pattern, which – in turn – also determines masculinization of hepatic steroid metabolism (Jansson *et al.* 1985). Also, perinatal androgens appear a key determinant of adult bone length in males, as shown by the lower femoral and tibial length in androgen-deficient hypogonadal mice compared to age-matched orchidectomized mice (Sims *et al.* 2006). In addition, it is now well established, both in humans and animals, that estrogens may interact with the GH-IGF-I axis (Juul 2001, Venken *et al.* 2005). In fact, estrogen-related changes in male bone mass seem to be IGF-I-dependent. In male mice, estrogen-dependent skeletal changes are associated with lower IGF-I levels during early puberty. Similar findings have been reported in ERαKO mice and male rats treated with an aromatase inhibitor (Vanderschueren *et al.* 1997, Vidal *et al.* 2000). Likewise, aromatase inhibition in adolescent boys decreases estrogen levels and is associated with a concomitant reduction of IGF-I levels (Mauras *et al.* 2000). Also, a recent study in cartilage-specific ERα KO mice showed that low $E_2$ levels during early skeletal maturation stimulate longitudinal bone growth through actions on the GH-IGF-I axis, independent of ERα in growth plate cartilage (Borjesson *et al.* 2010). Collectively, a significant body of evidence identifies GH/IGF-I as a critical regulator of skeletal gender differences. Nevertheless, the notion that sex steroids may

interact with IGF-I suggests that the androgens and estrogens may indirectly affect the skeletal sexual dimorphism as well through interaction with GH-IGF-I (Figure 1).

Overall, the development of skeletal sexual dimorphism in mice resembles the human situation, since most of the gender differences in bone mass are established during puberty and associated with similar hormonal changes (Figure 1). Nevertheless, some differences become apparent as well. In humans, it is generally accepted that sex differences in bone morphology are the result of the earlier onset of puberty in girls and the longer duration of puberty in boys, without major differences in absolute growth rate between sexes (Seeman 2002, Iuliano-Burns *et al.* 2009). In mice on the other hand absolute gender differences in periosteal and endocortical bone formation also contribute to the development of the skeletal sexual dimorphism (Callewaert *et al.* 2010b). In fact, periosteal and endosteal bone formation rates as determined by dynamic histomorphometry are higher and lower, respectively, in male versus female mice. Obviously, similar information on dynamic bone formation rates in humans is not available. Differences with respect to pubertal GH-IGF-I secretion may exist between humans and mice as well. In contrast with mice, peak IGF-I levels are not different between boys and girls. However, girls have an earlier IGF-I peak associated with the earlier onset of puberty (Leger *et al.* 2007). Overall, extrapolation of mice data on hormonal determinants of skeletal sexual dimorphism to the human condition should be handled with caution.

**Relative importance of androgens and estrogens during male bone growth**

It has become increasingly clear that androgen signaling in males is far more complex than originally anticipated. A significant body of evidence in humans and animals has now firmly established that at least part of the androgen-mediated bone growth in males may be mediated through conversion of androgens into estrogens and subsequent ERα activation. In fact, aromatase-deficient men, estrogen-resistant men as well as transgenic mice lacking the aromatase or ERα gene all present with low bone mass (Vidal *et al.* 2000, Miyaura *et al.* 2001, Bouillon *et al.* 2004, Rochira *et al.* 2007, Smith *et al.* 2008). Together these findings support the view that estrogens are indispensable for male skeletal health. Nevertheless, more conclusive evidence on the relative importance and differential roles of AR and ERα in cortical and trabecular bone mass accrual and maintenance was only recently provided by the longitudinal evaluation of male mice lacking both AR and ERα (Callewaert *et al.* 2009). This study supports AR activation as the sole responsible for the development and maintenance of male trabecular bone mass, since AR inactivation – in the presence or absence of ERα – results in a severely reduced trabecular bone mass with increased bone turnover (Callewaert *et al.* 2009). Similar changes – also described in other AR knockout (KO) models (Kawano *et al.* 2003, Venken *et al.* 2006) – are main features of hypogonadal osteoporosis as well (Finkelstein *et al.* 1989). Along the same line, testosterone treatment restores orchidectomy-induced trabecular bone loss in male mice (Venken *et al.* 2006), adding further evidence to the concept of AR signaling as a critical regulator of the development of normal male trabecular bone mass. On the other hand, administration of an aromatase inhibitor in ARKO and orchidectomized mice has no effect on trabecular bone (Venken *et al.* 2006),

suggesting that residual ERα activation fails to restore or compensate AR-mediated bone loss. This lack of a role of ERα during male trabecular bone growth sharply contrasts with the important role of ERα in female mice (Lindberg *et al.* 2001), or with the pharmacological effect of (supraphysiological) estrogen administration in orchidectomized male mice (Vandenput *et al.* 2001). Moreover, various studies even report an increased trabecular bone mass in ERαKO mice (Vandenput *et al.* 2001, Sims *et al.* 2003, Callewaert *et al.* 2009), which could be attributed to higher androgen levels acting through the AR. In line with this assumption, surgical castration or antiandrogen treatment of ERαKO mice normalizes the trabecular bone mass in these mice (Vandenput *et al.* 2001, Sims *et al.* 2003). Together, these observations clearly define AR and ERα activation as the primary determinant of trabecular bone development in male and female mice, respectively.

In contrast with their role in trabecular bone, AR and ERα are both required for optimal stimulation of male cortical bone mass in male mice. In fact, AR-ERα 'double' KO mice have lower cortical bone mass compared with either ARKO or ERαKO mice alone (Callewaert *et al.* 2009). Likewise, administration of an aromatase inhibitor further reduces cortical bone mass in orchidectomized mice (Callewaert *et al.* 2010b). In line with these findings, testosterone action on periosteal bone formation and cortical thickness is blunted by an aromatase inhibitor in orchidectomized mice (Venken *et al.* 2006). The importance of aromatization of androgens into estrogens for cortical bone expansion is also supported by observations in humans. Cortical bone dimensions failed to enlarge in an adolescent aromatase-deficient boy despite supranormal testosterone concentrations (Bouillon *et al.* 2004). In this patient, estrogen treatment substantially

increased bone size, suggesting that optimal cortical bone expansion requires activation of both AR and ERα, not only in mice but also in humans. Beside ERα, estrogens might also activate ERβ. In contrast with female ERβKO mice – which show an increased cortical bone mineral content and cross-sectional area – ERβ does not appear to play any role in male skeletal growth, since male ERβKO do not display a bone phenotype (Windahl *et al.* 1999, Vidal *et al.* 2000, Sims *et al.* 2002). Thus, ERα but not ERβ appears to mediate estrogen actions during male skeletal growth. Although AR activation is the dominant pathway of androgen signaling for male trabecular bone growth and maintenance, it would seem therefore that AR and ERα activation are both required to optimize cortical bone growth (Figure 2).

**Androgen signaling and the mechanical sensitivity of the male skeleton**

*Functional response of bone to loading*

Mechanical loading has a major impact on skeletal growth. The change in body weight and resulting mechanical stimulation of the skeleton during puberty are substantial. Since overall growth rate is higher in males than females, the male skeleton encounters higher mechanical demands. It is therefore tempting to speculate that this higher load-bearing in males also stimulates bone growth to a greater extent than in females, as reflected by more periosteal bone formation during puberty (Callewaert *et al.* 2010b). The stimulatory role of mechanical loading and physical activity on bone expansion has been well documented in numerous experimental and clinical studies. A range of non-invasive axial

loading models, using various animal models subjected to different exercise regimens, have provided insights in the anabolic response of bone to loading (Mosley *et al.* 1997, Mosley & Lanyon 1998, Hsieh & Turner 2001, Srinivasan *et al.* 2002). Overall, these animal studies have consistently indicated that mechanical loading influences the morphology of growing bone by increasing bone formation more than resorption. In humans, most evidence is derived from cross-sectional observations. For instance, tennis players have been shown to have larger cortical thickness in the dominant playing arm compared with the nonplaying arm (Bass *et al.* 2002). Moreover, the increase in bone mass in elite gymnasts persists after retirement, suggesting that the beneficial effects of loading may induce lifelong benefits to bone strength (Bass *et al.* 1998). Similar findings have been obtained in a few longitudinal studies, reporting significant side-to-side differences in bone size and strength in tennis players 1.5-3 years after retirement (Haapasalo *et al.* 2000). Yet, the assumption that skeletal benefits obtained from exercise may be maintained into older age remains uncertain, as unequivocal longitudinal evidence is currently lacking. The timing and duration of exercise also influences the response to loading in humans. In fact, different responses to loading have been reported in pre-, peri- and postpubertal tennis players. In female players, loading before puberty and during early puberty enhances periosteal bone formation and bone strength, whereas loading during late puberty bone is mainly accumulated at the endocortical bone surface without major changes in bone strength (Bass *et al.* 2002). In male players, on the other hand, periosteal expansion in response to loading is most pronounced in pre- and peripubertal boys and then tends to plateau (Ducher *et al.* 2009). It would seem therefore

that pubertal growth and sex hormones appear to influence the adaptive response to exercise, and this interaction may be different in boys and girls.

*Interaction of sex steroids and mechanical loading*

Sex steroids may also modulate the response of bone to mechanical stimulation. This hypothesis originates from rodent studies, showing that female rats have more bone than male rats relative to body weight (Saville 1969, Wang *et al.* 2003). A study in human observed a similar evolution, as the increase in bone mass relative to muscle mass is greater in girls compared with boys during puberty (Schiessl *et al.* 1998), even after adjustment for fat mass (Ferretti *et al.* 1998). These observations suggest that estrogens alter the mechanosensitivity of bones, so that more bone is accumulated than is needed mechanically. Moreover, it has been hypothesized that estrogen withdrawal during menopause impairs the mechanically adaptive mechanism, and hereby contributes to postmenopausal bone loss and the development of osteoporosis (Lanyon & Skerry 2001). Exercise, however, is generally believed to stimulate periosteal bone formation, whereas estrogen appears to have an inhibitory effect in females – at least in mice (Callewaert *et al.* 2010b). For instance, prepubertal female tennis players with low estrogen concentrations show a more favorable periosteal response compared to postpubertal girls with high estrogen levels (Bass *et al.* 2002). Concurrently, estrogen supplementation in male rats appears to suppress the periosteal response to mechanical loading (Saxon & Turner 2006). According to *in vitro* as well as *in vivo* rodent studies, sex steroid signaling and mechanical loading may also share common signaling pathways. Estrogen and

mechanical strain stimulate proliferation independently in osteoblast-like cells derived from male and female rat as well human osteoblasts (Damien *et al.* 2000, Cheng *et al.* 2002). Nevertheless, estrogen receptor modulators and antagonists block the increase in proliferation in response to mechanical strain, whereas DHT and AR activation apparently are not involved. These findings suggest that ERs, but not AR, influence the response to strain. As a proof of this concept, periosteal bone formation in response to *in vivo* ulna loading is significantly reduced in female ERαKO mice (Lee *et al.* 2003). In contrast, disruption of ERβ increases periosteal bone formation following loading in female but not male mice (Saxon *et al.* 2007). Together, these findings clearly indicate that sex hormone signaling interferes with the mechanical response to loading, at least in female mice, with ERα and ERβ having antagonistic effects. A recent report also investigated the role of sex steroid receptors in the mechanical sensitivity of the male skeleton (Callewaert *et al.* 2010c). This study suggests that ERα does not interfere with the adaptive response in males, which is in sharp contrast with the above-mentioned crucial role of ERα in females. Indeed, periosteal bone formation is similarly increased following loading in male ERαKO compared with WT mice. The apparent gender-dependent importance of ERα in mice is also supported by observations in humans, since puberty and sex steroid exposure influence the mechanical bone response differently in boys and girls (Bass *et al.* 2002, Ducher *et al.* 2009). In addition, AR activation may even limit the response to mechanical stimulation, since AR deletion – both in the presence and absence of ERα – increases the periosteal bone response to loading (Callewaert *et al.* 2010c). AR signaling not only increases periosteal bone formation following loading, but also lowers SOST and sclerostin expression (Callewaert *et al.* 2010c). Sclerostin, which

is encoded by the SOST gene, is the principal osteocyte-specific inhibitor of bone formation known to antagonize Wnt/β-catenin signaling (Poole *et al.* 2005, ten Dijke *et al.* 2008). The role of osteocyte-specific sclerostin signaling in mechanotransduction has previously been demonstrated in both loading and unloading conditions (Robling *et al.* 2008, Lin *et al.* 2009). Therefore, AR signaling may interfere with the inhibiting effect of sclerostin signaling on bone formation. In conclusion, *in vitro* and *in vivo* animal data as well as observations in humans have indicated that estrogen may modulate the effects of loading in females. In fact, ERα activation is required for the full osteogenic response to loading, whereas ERβ activation appears to inhibit periosteal bone formation following loading in females. In contrast with female mice, ERα and ERβ apparently are not involved in the response to loading in male mice. AR signaling, on the other hand, limits the anabolic response to mechanical loading in male mice, possibly through interaction with sclerostin signaling (Figure 3).

## Conclusion

GH/IGF-I action, more than sex steroid action, appears to be the primary determinant of gender differences in pubertal bone growth, which – in turn – may be influenced by sex hormones (*e.g.* neonatal imprinting). Beside GH/IGF-I, estrogens limit periosteal bone expansion but stimulate endocortical apposition in females. In males, both androgens and estrogens stimulate periosteal bone expansion and hence cortical bone growth during puberty. However, estrogen action on male cortical bone may at least partly be explained by concomitant changes in IGF-I (Figure 1). In contrast with its role in cortical bone growth, AR activation alone is sufficient for the development of trabecular bone mass in males (Figure 2). Finally, sex steroids and their receptors also impact on the mechanical sensitivity of the male and female skeleton. AR and ERβ signaling limit the osteogenic response to mechanical loading in male and female mice, respectively. ERα activation, on the other hand, stimulates bone formation in response to mechanical loading in female but not male mice (Figure 3). Therefore, skeletal sexual dimorphism is not only determined by androgen action in males and estrogen action in females, respectively, but also by complex gender- and time-specific interactions between sex hormones, GH-IGF-I and mechanical loading.

## Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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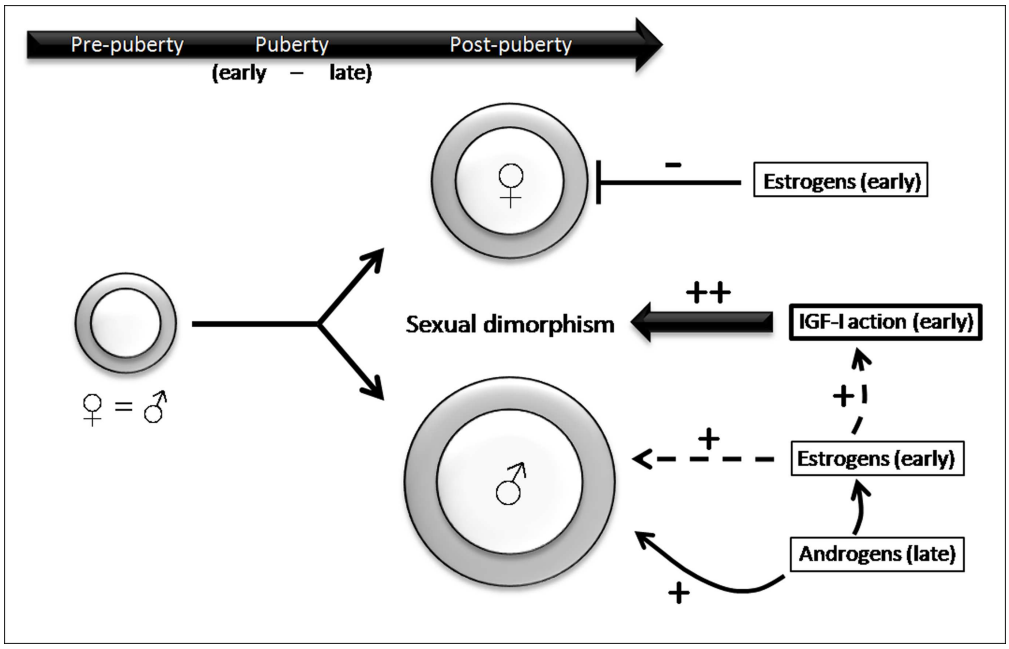
**Figure legends**

**Figure 1**. Insulin-like growth factor-I (IGF-I) is the primary determinant of the skeletal sexual dimorphism, which develops during puberty. Male androgen action also contributes to the larger male bone size by stimulating radial bone expansion mostly during late puberty. Male estrogen action has early stimulatory effects on periosteal bone expansion, but may be regulated through changes in IGF-I levels. In female mice estrogens limit radial bone expansion already during early puberty.

**Figure 2**. Differential roles of AR (androgen receptor) and ERα or –β (estrogen receptor-α or -β) signaling in the accrual of an optimal cortical and trabecular bone mass in male and female mice. (T = testosterone; $E_2$ = 17β-estradiol)

**Figure 3**. Interaction of sex steroids and their receptors with the adaptive response of bone to mechanical stimulation. In female mice, ERα activation is required for the full osteogenic response to loading, while ERβ activation inhibits it. In contrast, ERα or ERβ activation in male mice seems not important for an optimal response to mechanical stimulation. AR activation on the other hand limits the mechanical sensitivity of the male skeleton, potentially through downregulation of SOST signaling. ($E_2$ = 17β-estradiol; T = testosterone; ERα = estrogen receptor-α; ERβ = estrogen receptor-β; AR = androgen receptor)

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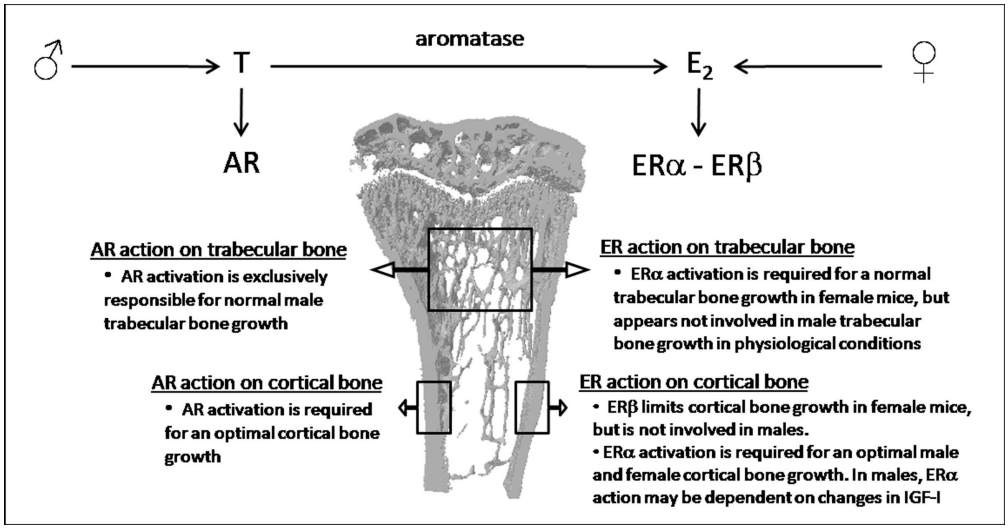


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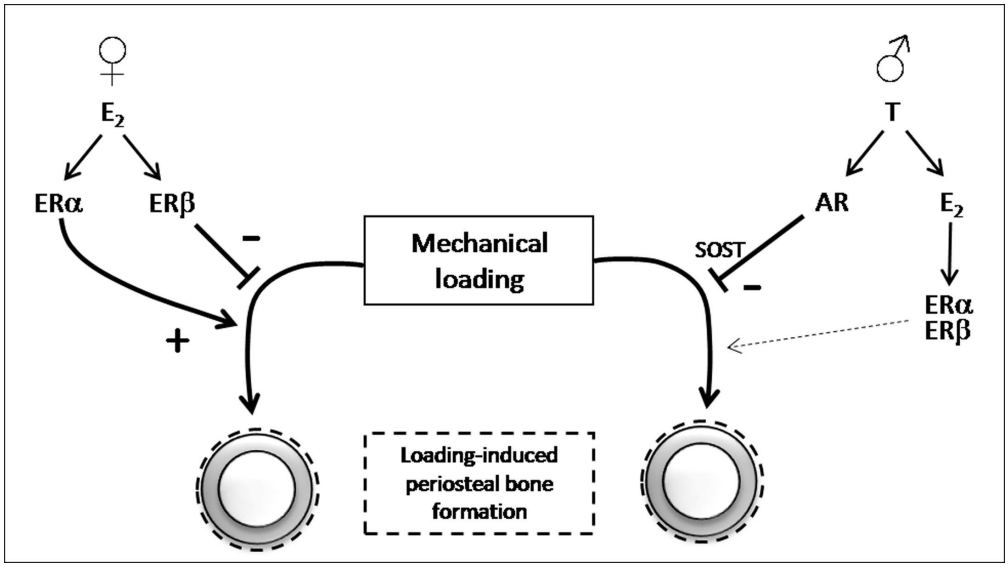


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